## AMENDMENT TO THE SPECIFICATION

Please amend the title as the following:

CRYSTALLIZATION OF MVAS (HMG-COA SYNTHASE)

Please amend the paragraphs [0054], [0090], [00178], [00179], and [00183], Tables 2-4 on page 14-15 in the specification as the followings:

[0054] For example, based on the crystal structure, applicants have determined that the MvaS amino acids shown in Table 2 encompass a 4-Angstrom radius around the MvaS (SEQ ID NO:1) active site and thus likely to interact with any active site inhibitor of MvaS. Applicants have also determined that the amino acids of Table 3 encompass a 7-Angstrom radius around the MvaS (SEQ ID NO:1) active site. Further it has been determined that the amino acids of Table 4 encompass a 10-Angstrom radius around the MvaS active site. It is noted that there is one MvaS molecule in the asymmetric unit, referred to as chain A. Structural coordinates appear in Figure 3. It is noted that the sequence and structure of the residues in the active site may also be conserved and hence pertinent to other MvaS and homologs.

**Table 2**: Amino Acids encompassed by a 4-Angstrom radius around the MvaS (SEQ ID NO:1) active site.

| | | |
|---|---|---|
| ASP 29 | GLY 31 | LYS 32 |
| GLY 36 | GLU 79 | ALA 110 |
| CYS 111 | TYR 143 | GLY 148 |
| GLY 149 | THR 152 | PHE 185 |
| VAL 196 | GLY 198 | SER 201 |
| ASN 202 | TYR 205 | HIS 233 |
| PRO 235 | TYR 236 | MSE 239 |
| LYS 242 | ASN 275 | TYR 306 |
| GLY 307 | SER 308 | |

**Table 3**: Amino Acids encompassed by a 7-Angstrom radius around the MvaS (SEQ ID NO:1) active site.

| | | |
|---|---|---|
| VAL 28 | ASP 29 | PRO 30 |
| GLY 31 | LYS 32 | PHE 33 |
| HIS 34 | ILE 35 | GLY 36 |
| ILE 37 | GLU 79 | GLU 109 |
| ALA 110 | CYS 111 | TYR 112 |
| ASP 139 | TYR 143 | SER 147 |
| GLY 148 | GLY 149 | GLU 150 |
| PRO 151 | THR 152 | ASP 184 |
| PHE 185 | TRP 186 | PRO 194 |
| VAL 196 | ASP 197 | GLY 198 |
| PRO 199 | LEU 200 | SER 201 |
| ASN 202 | GLU 203 | THR 204 |
| TYR 205 | ILE 206 | HIS 233 |
| ILE 234 | PRO 235 | TYR 236 |
| LYS 238 | MSE 239 | LYS 242 |
| ASN 275 | TYR 277 | THR 278 |
| SER 280 | TYR 306 | GLY 307 |
| SER 308 | GLY 309 | |

**Table 4**: Amino Acids encompassed by a 10-Angstrom radius around the MvaS (SEQ ID NO:1) active site.

| | | |
|---|---|---|
| MSE 19 | ALA 23 | ASN 27 |
| VAL 28 | ASP 29 | PRO 30 |
| GLY 31 | LYS 32 | PHE 33 |
| HIS 34 | ILE 35 | GLY 36 |
| ILE 37 | GLY 38 | GLN 39 |
| MSE 42 | THR 78 | GLU 79 |
| SER 80 | LYS 108 | GLU 109 |
| ALA 110 | CYS 111 | TYR 112 |
| GLY 113 | ALA 114 | ASP 139 |
| ILE 140 | ALA 141 | LYS 142 |
| TYR 143 | GLY 144 | ASN 146 |
| SER 147 | GLY 148 | GLY 149 |

| | | |
|---|---|---|
| GLU 150 | PRO 151 | THR 152 |
| GLN 153 | GLY 154 | ILE 182 |
| TYR 183 | ASP 184 | PHE 185 |
| TRP 186 | ARG 187 | PRO 194 |
| MSE 195 | VAL 196 | ASP 197 |
| GLY 198 | PRO 199 | LEU 200 |
| SER 201 | ASN 202 | GLU 203 |
| THR 204 | TYR 205 | ILE 206 |
| GLN 207 | SER 208 | PHE 232 |
| HIS 233 | ILE 234 | PRO 235 |
| TYR 236 | THR 237 | LYS 238 |
| MSE 239 | GLY 240 | LYS 241 |
| LYS 242 | ALA 243 | LEU 245 |
| TYR 263 | GLY 274 | ASN 275 |
| LEU 276 | TYR 277 | THR 278 |
| GLY 279 | SER 280 | LEU 281 |
| PHE 304 | SER 305 | TYR 306 |
| GLY 307 | SER 308 | GLY 309 |
| ALA 310 | VAL 311 | ALA 312 |

[0090] By performing submicroliter volume sized crystallization experiments, as detailed in U.S. Patent No. 6,296,673, effective crystallization conditions for forming crystals of a MvaS complex were obtained. In order to accomplish this, systematic broad screen crystallization trials were performed on an MvaS complex using the sitting drop technique. In each experiment, a 100nL mixture of MvaS complex and precipitant was placed on a platform positioned over a well containing ~~100□L~~ 100μL of the precipitating solution. Precipitate and crystal formation was detected in the sitting drops. Fine screening was then carried out for those crystallization conditions that appeared to produce precipitate and/or crystal in the drops.

[00178] The gene encoding residues 1-383 (from ~~SEQ. ID No. 1~~ SEQ ID NO:1), which corresponds to the full-length MvaS from E. faecalis, was isolated by PCR from E. faecalis genomic DNA (ATCC700800D ) and cloned into the TOPO-activated cloning site of pSX26 vector. This DNA sequence is presented in ~~SEQ. ID No. 2~~ SEQ ID NO:2. Expression in this vector generated a fusion of the full-length MvaS with non-cleavable carboxy-terminal six

histidine tag, the amino acid sequence of which is shown, underlined, in Figure 1 (~~SEQ. ID No. 1~~ SEQ ID NO:1). For production of seleno methionine labeled protein, the expression plasmid encoding for MvaS_Ef fused with carboxy-terminal histidine tag was transformed into methionine auxotroph DL41 (Hendrickson, W. 1990. EMBO J. 9:1655-0).

[00179] Biomass for purification of recombinant seleno-methionine labeled MvaS_Ef was generated using minimal media supplemented with seleno-methionine (Sigma, MO) using 96-well fermentor. It should be noted that a variety of other protocols and expression strains are also suitable for the expression of selenomethionine derivative of MvaS, website: cbr.med.harvard.edu/investigators/springer/lab/protocols/sara_SeMet.html (~~http://cbr.med.harvard.edu/investigators/springer/lab/protocols/sara_SeMet.html~~); Doublie, S. (1997)[[.]], *Methods in Enzymology* 276, 523-530; website: novagen.com/docs/ndis/INNO10-005.pdf ~~http://www.novagen.com/docs/ndis/INNO10-005.pdf~~), as would be readily appreciated by one of skill in the art. Cells from a single 70 ml fermentor tubes was thawed by addition of 21 ml of lysis buffer (50 mM Tris/HCl pH 7.9, 50 mM NaCl, 1 mM $MgCl_2$) containing hen egg white lysozyme (0.6 mg/ml) and Benzonase (2.5 U/ml) and sonicated using Sonic Hedgehog robot. The sonicate was allowed to stand for 30 minutes at ~4°C. Total lysate was clarified by centrifugation and 2mL of 5M NaCl were added to the cleared lysate. The cleared lysate from four fermentor tubes was applied to 3 ml bed ProBond column that had been equilibrated to 50 mM Potassium Phosphate pH 7.8, 0.4 M NaCl, 0.1 M KCl, 20 mM imidazole, 10% glycerol, 0.25 mM TCEP. The solution was passed through the column using gravity flow and the column was washed with 6 bed volumes of 50 mM Potassium Phosphate pH 7.8, 0.4 M NaCl, 0.1 M KCl, 40 mM imidazole, 10% glycerol, 0.25 mM TCEP (Tris(2-carboxyethyl)phosphine hydrochloride). The product was eluted with 12 ml of 50 mM Potassium Phosphate pH 7.4, 0.4 M NaCl, 0.1 M KCl, 200 mM imidazole, 10% glycerol, 0.25mM TCEP. The eluted protein was concentrated and buffer-exchanged into 25 mM Tris pH 7.9, 150 mM NaCl by using Vivaspin centrifugal concentrators. Following three five-fold dilution buffer-exchanges, the IMAC (immobilized metal affinity chromatography, Clontech, Mountain View, CA) purified MvaS_Ef

was concentrated to 10 mg/ml with a total volume of 0.47 ml. The molecular weight of the purified protein corresponded to the 100% incorporation of seleno-methionine as determined by Mass Spectrograph (MS) analysis (43,443 observed and 43,436 expected without N-terminal methionine). Purified MvaS_Ef exhibited a major band by both isoelectric focusing (IEF) and by sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analyses.

[00183] Provided are crystals relating to MvaS (HMG-CoA Synthase) and its various uses.